International Journal of Mass Spectrometry 345–347 (2013) 8–18

Contents lists available at SciVerse ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

The effect of salt on the conformations of three model proteins is revealed by variable temperature ion mobility mass spectrometry

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ARTICLE INFO

Article history:
Received 24 June 2012
Received in revised form 30 January 2013
Accepted 8 February 2013
Available online 26 February 2013

Keywords:
Ion mobility mass spectrometry
Hofmeister effect
Cold denaturisation

ABSTRACT

Three 'model' proteins of varying molecular mass: lysozyme, cytochrome C, and BPTI are incubated with sodium iodide and following nano-spray ionisation, their gas-phase conformations are determined using drift tube ion mobility mass spectrometry (DT IM-MS). Ion mobility measurements were carried out on these proteins with helium as the buffer gas at three different drift cell temperatures – 'ambient' (300 K), 'cold' (260 K) and 'hot' (360 K). Significant levels of adducted iodide are observed on all three proteins with the number of iodides correlating to the number of available basic sites. The conformational space occupied by each protein is altered in the presence of salt, this is exhibited by a reduction in the intensities of adducted species for high and low charge states for each protein. This 'salting in' reduces the CCS spread for lysozyme and BTPI to converge on the values calculated from the crystal structure data. A change in conformation of all proteins is observed as a function of charge state, which is attributed to coulombically driven unfolding. Thermally induced unfolding (which is observed at both cold and hot temperatures) is minimised in the presence of adducted iodide. This is the first report of 'cold denaturisation' for proteins in the gas phase and suggests that this effect maybe intrinsic to the protein fold.

1. Introduction

Protein–salt interactions play a key role in the structure of a protein and on stabilising its active form. Franz Hofmeister was the first to consider how salt ions affect proteins, and this work culminated in the 1888 milestone publication 'Zur Lehre von der Wirkung der Salze' (About the Science of the Effect of Salts) [1]. He established that these effects vary widely, however some trends could be established for cations and anions separately. These trends were the salts' ability to precipitate proteins, which gave rise to the Hofmeister series–which contained a separate ranking for cations and anions. Recent years have seen a revival of curiosity in this topic with a more than 3-fold increase in citations in the past decade compared to the previous one. This increased interest in the Hofmeister effect is testament to its fundamental relevance to a wide range of fields: from enzyme activity [2] and protein stability [3] to protein–protein interactions [4] and protein crystallisation [5] and also to the development of new experimental tools [6] and models [7] that can help to shed light on this long established phenomenon. As a result of these recent research efforts, the initial hypothesis that the Hofmeister phenomenon is due to the effect of salts in making or breaking the structure of water has gradually fallen out of favour [8]. In its place has come an assertion that the key instigator of precipitation is via direct ion–macromolecule interactions. These interactions are very amenable to gas-phase studies.

Through introduction of soft ionisation techniques of MALDI [9,10] and ESI [11,12], mass spectrometry has become a popular technology underpinning protein research. Gas-phase protein ions [13] and their complexes [14–16] are now studied directly as a population of 'naked' ions [17], decoupled from any counter-ions. Volatile buffers are used to provide 'clean' mass spectra [18] although native-ESI mass spectrometry peaks are often somewhat broadened by the presence of un-desolvated buffer salts and/or water [19]. Further structural elucidation is enabled by collision induced dissociation (CID)[20], whereby proteins complexes are dissected into their constituent parts [21–23] by collisions with inert gas. However, in biological systems, proteins are never alone: they are crowded by other macromolecules and ions which play a crucial role in their stabilisation and function [24]. The focus of this work is to examine the effect of anions on proteins with mass spectrometry. In recent years, mass spectrometry based studies, and in particular those which aim to preserve biologically relevant conformations for gas-phase analysis, have gradually evolved from considering adducted salts as a regrettable feature to regarding them key to retaining a stable gas-phase fold [25,26].

IM-MS provides structural information on gas-phase ions [27] and has been applied to the analysis of both specific and non-specific interactions between ions and proteins [25,28–30] Drift

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http://dx.doi.org/10.1016/j.ijms.2013.02.005

tube ion-mobility mass spectrometry instrumentation (DT IM-MS) can be used to determine the time it takes a pulse of ions to pass through a drift cell containing an inert gas under the influence of a weak electric field [31]. In such instruments, the mobility (K) of a given ion is defined as the constant of proportionality between the velocity at which it drifts (v_d) and the applied electric field (E). To allow inter-laboratory comparisons, it is usual to standardise the mobility to 273.15 K and 760 Torr to the reduced mobility (K_0), which is inversely proportional to the collision cross section (Ω) of the gas-phase ion:

$$K_0 = \frac{3ze}{16N}\left(\frac{2\pi}{\mu k_B T}\right)^{1/2}\frac{1}{\Omega} \quad (1)$$

where z is the ion charge state, e is the elementary charge, N is the gas number density, μ is the reduced mass of the ion-neutral pair, k_B is the Boltzmann constant and T is the temperature of the buffer gas.

In this work we use ion mobility mass spectrometry to examine how the addition of iodide, and also temperature, affects the conformational stability of three model proteins of variable molecular mass: lysozyme, cytochrome C and BPTI. We interpret this data in terms of the intrinsic effects of anion addition on protein fold.

It has been shown recently that anions attached to proteins tend to stabilise compact conformations [26] and that the number of certain anions (such as perchlorate and iodide) that can adduct to a protein, correlates well with the number of available basic sites [32]. It follows from this that a protein surface can be 'mapped' by such anions. Sodium iodide was chosen for this study for a number of reasons. Iodide is a chaotropic anion in the Hofmeister series [1] and, along with other ingredients, is a pharmaceutically acceptable anion (i.e., used in formulations of medicines) used to stabilise biomolecules (proteins, DNA, RNA) in biological matrices (blood, saliva, urine etc.) for their prolonged storage and shipment [33]. Iodide has been shown to have a destabilising effect on protein complexes [26], as it can bind to non-polar patches on proteins, in addition to cationic residues [34]. Of all halides, iodide has the largest ionic radius (disregarding astatine, which is unstable and radioactive, and hence pharmaceutically unsuitable) [35], measuring to ~2.4 Å [36,37] and therefore the lowest charge density. For this reason, this anion will bind to proteins more tightly [5] and any anticipated resulting conformational changes will be more profound and easier to observe. Finally, anions generally have a larger effect on proteins than cations [8,25,38] and so for this preliminary gas-phase study may provide more insight.

All three proteins investigated here are well characterised by other biophysical methods and therefore are good models for this study. Their crystal structures were first published in the 1970s and 1980s, and to date, more than 560 structures are available for lysozyme, over 200 for cytochrome C and approaching 100 for BPTI [39].

2. Experimental

2.1. Materials

Lysozyme from chicken egg white (product number L6876), cytochrome C from equine heart (product number C7752) and Bovine pancreatic trypsin inhibitor, BPTI (product number T0256) were purchased from Sigma–Aldrich Company Ltd. (Dorset, UK). 1 mM stock solution of each protein was prepared in deionised water (18 MΩ) and the aliquots were stored at −20 °C. For CCS measurements, 50 μM concentrations of each protein were prepared in deionised water with addition of 40-fold molar excess of sodium iodide (2 mM); these solution conditions were selected as being optimal over buffered aqueous solution (see supplementary material Figure S1). The pI of all of these model proteins is around 9 (lysozyme 9.32, cytochrome C 9.52, and BPTI 9.24) the final pH of the solutions after addition of sodium iodide, was 7.

2.2. Drift tube IM-MS measurements

DT IM-MS experiments were performed on a quadrupole time-of-flight mass spectrometer that has been modified in-house to include a 5.1 cm copper drift cell [40]. Ions were produced by positive nESI ionisation using a *Z*-spray source, within a spray voltage range of 1.2–1.8 kV and a source temperature of 80 °C. The effect of source temperature was investigated and it was found that there was no change in adduct levels between 60 and 100 °C (see supplementary material Figure S2).

The drift cell was filled with helium as the buffer gas and the pressure was measured using a capacitance manometer (MKS Instruments, UK) and was ~3.5 Torr for these experiments, the precise pressure was recorded for each and every drift voltage and used to evaluate the ion mobility and the corresponding collision cross section. Ions were injected into the cell with an energy of 33–38 V. The cell is heated via tungsten wire wound ceramic heaters located in both the cell body (8 heaters) and in the end cap (2 heaters) driven by Variacs, and cooled with pre-cooled dry nitrogen, passing though the channels in the cell body and end caps. Ion mobility measurements were carried out on these proteins at three different drift cell temperatures – 'ambient' (300 K), 'cold' (260 K) and 'hot' (360 K) to probe conformational dynamism of the proteins. The temperature of the drift cell was monitored and recorded, and was stable to <0.1 K for each recorded data set at 300 K and to <0.4 K for each set at 260 and 360 K. The electric potential across the cell was varied from 12 to 2 V cm^{-1}. Ion arrival time distributions (ATDs) were recorded by synchronisation of the release of ions into the drift cell with mass spectral acquisition.

2.3. Ion arrival times measurements and CCS calculations

Collision cross-sections of the ion series of BPTI, cytochrome C and lysozyme were measured in triplicates and the mean values reported (supplementary materials Tables ST1–3). Resulting arrival time distributions present as complex profiles often featuring more than one coeluting peak (usually two, with tailing and fronting), making the assignment of the peak maxima ambiguous and CCS calculation unreliable. Also, assigning one or two discrete CCS values to a vastly populated conformational domain would not reflect the physical reality of the events in the drift cell, as the results would be 'over-interpreted'. Therefore, the data need to be presented in their more 'native' form, i.e., as ATD profiles and at the same time, a comparison of the results obtained at different experimental conditions has to be possible. To enable this, the ion arrival time t_a scale needs to be converted to a CCS scale (via drift time t_d), for which the dead time value t_0 has to be known. A new method of Gaussian curve fitting has been developed to simultaneously fit the whole drift voltage series for a particular ion at once. Apart from improved data workup efficiency, this method also: (i) takes into account the interdependency of the ATD parameters within one drift voltage series; (ii) yields the dead time value t_0 associated with each of the m/z values, yielding mobilities, and hence CCS (via Equation 1 above), at known drift voltages. The Origin graphing software enables the user to define their custom functions, therefore the existing Gaussian fitting function has been modified to enable fitting the two-ion flux at specified drift voltage series. Resulting values of dead time t_0 and were used to convert arrival times into CCS. All the data obtained at 300 K were treated using this method, and the resulting mean values of t_0 were used to process the rest of the data obtained